A FEW WORDS ON OPSONINS*

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I have been asked by the committee in charge of program to present a paper on opsonic index and its relation to medicine. The paper must of necessity be but a compilation drawn from other men's work. I am chiefly indebted to my friend, Dr. G. W. Ross, for any knowledge I have on the subject. As this association exists essentially for clinical demonstrations, and as I am not doing the opsonic work myself at present, Dr. Mace has kindly consented to show you the various steps in the technic, so I will not touch on this side of the subject.

The fact that defense of the body against bacterial attack is a complicated and multiple one has long been known to pathologists. Erlich put forward the theory that where bacterial intoxications take place, the toxins of the invading organisms must enter into chemical combination with the cells of the different tissues. There are many toxins which have specific selective power; some on nerve, some on muscle, and undoubtedly some, which are less well known, such as poisons of mumps and leukemia, on lymphatic and gland tissues. While Erlich's theory will explain the processes of immunity to toxins such as eventuate in diphtheria and tetanus, it does not explain how different bacterial invasions are combatted. We know that the cell poisons produce in the body a number of antibodies which Wright has called antitropines, and these are protective each against the poison which produces it. Among the antitropines we have the well-known diphtheretic antitoxin and the bodies which cause the agglutination of bacilli on which the Widal reaction is based; others called precipitins which throw out of solution toxins in combination with foreign albumens; the lysins, which have the effect of dissolving invading organisms; and last, the opsonins, of which we are to speak more fully.

Metchnikoff for many years, in fact, since about 1890, has been maintaining that immunity in all its forms is entirely dependent upon the phagocytic power of the white blood corpuscles. Erlich, while accepting the fact that the phagocyte plays some role in the production of immunity, has also been strong in urging that it was but a minor one. For many years the Russian pathologist claimed that the white cell was the only factor in the phagocytic process and the only agent in the process of immunity, but the work done by Erlich and by his own followers has forced him to admit the presence of antitropic substances in the body fluids, although he believes that these antitropines are secretions or excretions of the white cells.

In 1903 A. W. Wright, who was then the Chief of the Pathological Laboratory of the British Army, and Douglass, presented to the Royal Society a paper in which they showed the following facts: First, that the white blood corpuscles, free from all serum, had no power to ingest staphylococci. Second, that the addition of a small amount of blood serum to the staphylococci caused the bacilli to be readily ingested by the leukocytes. Third, that the substance in the blood serum that enabled this phagocytosis to take place was themo-labile and became impotent on being heated 60 degrees C. Fourth, that the serum did not act upon the white blood cells, but upon the streptococci. Fifth, that the plasma and the blood serum acted equally well; and they concluded from these experiments that the power of phagocytosis rested not in the white corpuscle, but in the reaction of the substance found in the serum to the invading organism. In their communication to the Royal Society they chose to call this substance opsonin from the Greek word signifying "I prepare food for," with the idea that the name should be illustrative of the power this substance has of making the organism fit for ingestion by the leukocyte.

The first work, as I have said, was done on staphylococci, and it was shown that in cases of general staphylococcic infection, furunculosis, sycosis, carbuncle, and in fact any local staphylococcic infection, that the amount of opsonin present was markedly diminished. The plan was devised of comparing the power of phagocytosis in healthy corpuscles from the same individual when activated respectively by a pool of serum from several healthy persons and serum from an individual with a staphylococcic infection. The average number of staphylococci ingested by healthy leukocytes when activated by healthy serum from a large number of individuals was found to vary very little, provided always that the conditions of the experiment were the same. This ingestion of staphylococci was taken as the standard and the ingestion of staphylococci by the same corpuscles when activated by the serum of an infected individual was compared with it, and the resulting quotient represented the opsonic power of the blood of that individual for the staphylococci. This quotient is what is known as the opsonic index. Wright developed his methods by working with staphylococcus because it is the easiest of all the organisms to deal with under the circumstances demanded. But he soon carried the work further and showed that the opsonins are produced in the blood by the invasion of other organisms. In fact, the only ordinary pathogenic organism, I believe, for which he has not been able to find an opsonin is the diphtheria bacillus.

The most important application, so far, of this method of determining resistance of the body to infection, is in relation to tubercular infection.

Wright and Douglas, Bullock and Atkin, Ross and Freeman, and within the last year many workers in this country, especially Hektoen and Potter, have busied themselves with developing a method of making these determinations applicable to tubercular patients.

The technical difficulties are great. There is great difficulty in getting proper cultures of the tubercle bacillus. There is difficulty when one has the proper culture in preparing an emulsion of the tubercle bacillus which will not clump, and much ingenuity has been exercised in overcoming this.

Curiously enough, in tuberculosis the opsonic index may be either very high or very low, de-

* Read at the Polyclinic Gathering.

pending upon whether the infection is local or systemic. Local tuberculosis such as lupus, glands, early tuberculosis of the lungs, early tuberculosis of the bone, show a low index. The late tuberculosis of the lung shows a variable index, sometimes as high as two or three times the normal; at others equally low. The practical point in all this work lies in the relation of the opsonins to treatment. Wright and Ross and Bullock, treating large numbers of cases of different kinds of tubercular infections by inoculation methods controlled by opsonin determinations, have had much success. The same is true of Hollister in this country.

The idea of treatment is to increase the opsonins in the patient's blood so that the bacteria meeting these will become an easy prey to the white cells which have heretofore not been able properly to perform their work of phagocytosis. The method has been especially successful in cases of lupus. The plan is as follows:

The patient's opsonic index is determined: he is then given a measured amount of a vaccine composed of the dead and sterilized bodies of tubercle bacillus. These dead cultures are readily obtainable in the market in the form of Koch's tuberculin R. You will say that Koch's tuberculin antedates the opsonic theory and vaccinations with tuberculin R., have often proven disastrous to patients; this is very true, but any very powerful drug used empirically may become destructive to patients. The great value of Wright's method is that he is enabled to measure the effects of the dose and to give the tuberculin in a rational as opposed to an empirical manner. Having determined the opsonic index of his patient, he inoculates not to exceed 1-1000 of a milligram of dry tuberculin and then he takes the opsonic index of that patient to the tubercle bacillus daily.

It is the invariable result of the first inoculation that the opsonic content drops very markedly and remains low for some time, varying with the different type of case and with the individual. The drop in the opsonic content Wright calls the negative phase, but this within a short time rises and goes well above the normal. Now if while the patient's opsonins are rapidly descending, a second injection is given there will be a still further decrease in the opsonic power and a further injection still may overwhelm the patient and make him unable to fight the invading process. But if the opsonic index is watched and the second inoculation is given while the opsonic index is rising above normal, there will be a still greater rise and the defensive power will become much increased and the patient as a rule will go on to a better condition. The value of the opsonic method is here evident as a control to the inoculation.

From the work that is being done we are coming to learn that not only tubercular and staphylococci infections may be combatted, but a case Ross has recorded of a chronic empyema due to pneumococcus which cleared up promptly under vaccinations with dead pneumococci, controlled by opsonic determinations to pneumococci, shows that this method will be valuable in localized pneumo-infections. Other work done leads us to hope that the chronic joint infections due to invasions by Neiser's coccus may also soon be efficiently treated.

In fact, there is every reason to hope that in vaccination controlled by opsonin determinations we have a therapeutic method that will give us power against many of the infections over which we now have no control. Besides the therapeutic gain, the diagnostic value is great as evidenced by a case of sycosis Wright reports in which, when he found the opsonic index high for staphylococcus, he tried it for tubercle and, finding that index low, was enabled to diagnose a tubercular sycosis and to cure it with tuberculin. Ross' case of pneumococcus infection is another, and I have no doubt cases of hip joint disease will be earlier and more positively diagnosed because of our ability to determine this index. It has already been shown that children of tubercular mothers have on the average an index but half that of those whose mothers are not infected, and that the index of bottle-fed infants against a number of bacteria is less than that of the breast-fed. And so we have explained rationally a well-known clinical fact. Undoubtedly we will soon test out the effect of many remedies on the activating power of the serum. It will be interesting to know whether the coloid metals gain their potency as agents in septicemia by a power to increase the opsonic activity of the serum. So high an authority as Welch has proclaimed Wright's discovery the greatest in medicine since Koch gave us knowledge of the tubercle bacilli and the means to isolate and study them. Personally I think that he understates the importance of the work, and that we have in the method of Wright and Douglas a technic that will overturn much of our therapy and supplant it with a method of treatment at once rational and efficient.

OPSONIC TECHNIC.*

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The method of estimating the opsonic content of blood serum elaborated by A. E. Wright of London is briefly as follows:

The washed white blood cells, or leukocyte cream, is prepared by drawing ten or fifteen drops of blood from a normal individual into a small centrifuge tube filled with a 1.5 per cent. sodium citrate solution in .85 per cent. sodium chloride, shaking thoroughly and centrifuging about five minutes. The citrate solution is pipetted off and .85 per cent. sodium cholride added, the tube thoroughly shaken and again centrifuged. The red cells, being heavier, are thrown to the bottom of the tube and on top of these the leukocytes are seen as a thin gray film. These washed corpuscles are pipetted off and placed in a watch glass.

The bacterial emulsion in the case of tubercle bacilli is made by removing the growth from a culture with a platinum loup and thoroughly grinding

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